NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 392

TOXICOLOGY AND CARCINOGENESIS STUDIES OF CHLORINATED WATER

(CAS NOS. 7782-50-5 and 7681-52-9)

AND CHLORAMINATED WATER

(CAS NO. 10599-90-3)

(DEIONIZED AND CHARCOAL-FILTERED)

IN F344/N RATS AND B6C3F$_1$ MICE

(DRINKING WATER STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.W. Bristol, Ph.D.
J.K. Dunnick, Ph.D.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.K. Haseman, Ph.D.
M.P. Jokinen, D.V.M.
M.M. McDonald, D.V.M., Ph.D.
G.N. Rao, D.V.M., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Southern Research Institute

Conducted studies and evaluated tissues

J.D. Prejean, Ph.D., Principal Investigator
D.R. Farnell, D.V.M., Ph.D.
H.D. Giles, D.V.M., Ph.D.
J.E. Heath, D.V.M.
C. Lindamood, III, Ph.D.
R.B. Thompson, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assessment

J.F. Hardisty, D.V.M., Principal Investigator
R. Brown, D.V.M., M.S.
B.F. Hamilton, D.V.M., Ph.D.

Integrated Laboratory Systems

Prepared quality assurance audits

J.C. Bhandari, D.V.M., Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides and prepared pathology reports for rats (9 January 1990)

M.A. Stedham, D.V.M., M.S., Chair
Pathology Associates, Inc.
R. Brown, D.V.M., Ph.D.
Research Path. Services, Inc.
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.P. Jokinen, D.V.M.
National Toxicology Program
E.E. McConnell, D.V.M.
Private Consultant
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program

NTP Pathology Working Group

Evaluated slides and prepared pathology reports for mice (11 January 1990)

R.M. Kovatch, D.V.M., Chair
Pathology Associates, Inc.
R. Brown, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
G. Burger, D.V.M.
R.J. Reynolds
M.P. Jokinen, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
B. Stuart, D.V.M., Ph.D.
Mobay Corporation

Biotechnical Services, Inc.

Prepared Technical Report

L.G. Cockerham, Ph.D., Principal Investigator
J.L. Elledge, B.A.
J.A. Gregan, M.A.
M.C. Hirrel, Ph.D.
K.D. Mencer, B.A.

CONTENTS

ABSTRACT

Cl_2	NaOCl	NH_2Cl
Chlorine	**Sodium Hypochlorite**	**Chloramine**
CAS No.: 7782-50-5 Molecular Weight: 70.9 gas	CAS No.: 7681-52-9 Molecular Weight: 74.4 solid	CAS No.: 10599-90-3 Molecular Weight: 51.48 colorless liquid

Chlorine and chloramine are used as disinfectants in water supplies to prevent the spread of waterborne diseases. The U.S. Environmental Protection Agency and the U.S. Congress, through the Safe Drinking Water Acts and Amendments, initiated studies to determine the most effective way to disinfect water supplies and, at the same time, minimize any potential long-term health effects associated with direct chemical exposure or indirect chemical exposure through the formation of by-products. As part of this evaluation, 2-year studies of chlorinated or chloraminated deionized charcoal-filtered drinking water were conducted in F344/N rats and $B6C3F_1$ mice to determine the potential toxicity and carcinogenicity associated with prolonged exposure and eliminate possible confounding effects of byproducts of chlorination.

Chlorinated Water Studies

Water containing 0, 70, 140, or 275 ppm chlorine (based on available atomic chlorine) was provided to groups of 70 F344/N rats or $B6C3F_1$ mice of each sex for up to 2 years. Groups of 10 rats or mice of each sex were predesignated for evaluation at 14 or 15 weeks and 66 weeks.

Survival at 2 years of rats and mice receiving chlorinated water was similar to that of the controls. Mean body weights of dosed male rats, high-dose female rats, and dosed mice were slightly lower than those of their respective control groups. There was a dose-related decrease in water consumption by rats and mice. Water consumption by high-dose rats during the second year of the studies was 21% lower than controls for males and 23% lower than controls for females; water consumption by high-dose mice was 31% lower than controls for males and 26% lower than controls for females.

The incidence of mononuclear cell leukemia in mid-dose, but not high-dose, female rats was significantly higher than that in controls (control, 8/50; low-dose, 7/50; mid-dose, 19/51; high-dose, 16/50). The proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all dose groups and controls. Although the marginal increase in leukemia incidence in the mid- and high-dose female rats suggested a possible association with the administration of chlorinated water, the incidence of leukemia was not clearly dose related. There was no indication of reduced latency of leukemia, and the incidence of leukemia in concurrent controls was less than the mean for historical controls; furthermore, there was no supporting evidence of an effect in male rats. Thus, the marginal increase in leukemia incidence in female rats was considered equivocal evidence of carcinogenic activity. There were no neoplasms or nonneoplastic lesions in male rats or in male or female mice that were clearly associated with the consumption of chlorinated water.

Chloraminated Water Studies

Water containing 50, 100, or 200 ppm chloramine was provided to groups of 70 F344/N rats or $B6C3F_1$ mice of each sex for up to 2 years. The same control groups were used for the chlorinated water and chloraminated water studies. Groups of 9 or 10 rats or mice of each sex were evaluated at 14 or 15 weeks and 66 weeks.

Survival at 2 years of rats and mice receiving chloraminated water was similar to that of the controls. Mean body weights of high-dose rats and dosed mice were lower than those of their respective control groups. There was a dose-related decrease in water consumption by rats and mice. Water consumption during the second year of the studies by high-dose rats was 34% lower than controls for males and 31% lower than controls for females; water consumption by high-dose mice was 42% lower than controls for males and 40% lower than controls for females.

Mononuclear cell leukemia occurred with a marginally increased incidence in the mid- and high-dose female rats receiving chloraminated water (control, 8/50; low dose, 11/50; mid dose, 15/50; and high dose, 16/50). As in female rats receiving chlorinated water, the proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all dose groups and controls. The marginal increase in leukemia incidence in females receiving chloraminated water was considered equivocal evidence of carcinogenic activity for the same reasons given for female rats receiving chlorinated water. There were no neoplasms or nonneoplastic lesions in male rats or in male or female mice that were clearly associated with the consumption of chloraminated water.

Conclusions

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity** of chlorinated water in male F344/N rats receiving 70, 140, or 275 ppm. There was *equivocal evidence of carcinogenic activity* of chlorinated water in female F344/N rats based on an increase in the incidence of mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of chlorinated water in male or female $B6C3F_1$ mice receiving 70, 140, or 275 ppm.

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of chloraminated water in male F344/N rats receiving 50, 100, or 200 ppm. There was *equivocal evidence of carcinogenic activity* of chloraminated water in female F344/N rats based on an increase in the incidence of mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of chloraminated water in male or female $B6C3F_1$ mice receiving 50, 100, or 200 ppm.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis Studies of Chlorinated Water

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 70, 140, or 275 ppm in buffered water	0, 70, 140, or 275 ppm in buffered water	0, 70, 140, or 275 ppm in buffered water	0, 70, 140, or 275 ppm in buffered water
2-Year survival rates	14/51, 6/51, 16/50, 17/51	31/50, 31/50, 28/51, 35/50	34/50, 28/50, 35/50, 32/51	33/50, 31/51, 28/50, 35/50
Body weights	Slightly lower than controls	High-dose slightly lower than controls	Slightly lower than controls	Slightly lower than controls
Water consumption	Mid- and high-dose less than controls	Dosed less than controls	Dosed less than controls	Dosed less than controls
Nonneoplastic effects	None	None	None	None
Neoplastic effects	None	Mononuclear cell leukemia (8/50, 7/50, 19/51, 16/50)	None	None
Level of evidence of carcinogenic activity	No evidence	Equivocal evidence	No evidence	No evidence

Summary of the 2-Year Carcinogenesis Studies of Chloraminated Water

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 50, 100, or 200 ppm in buffered water	0, 50, 100, or 200 ppm in buffered water	0, 50, 100, or 200 ppm in buffered water	0, 50, 100, or 200 ppm in buffered water
2-Year survival rates	14/51; 22/50; 14/51; 16/50	31/50; 28/50; 29/50; 24/50	34/50; 23/50; 34/50; 37/51	33/50; 32/50; 35/50; 42/50
Body weights	High-dose lower than controls	High-dose lower than controls	Dosed lower than controls	Dosed lower than controls
Water consumption	Dosed less than controls	Dosed less than controls	Dosed less than controls	Dosed less than controls
Nonneoplastic effects	None	None	None	None
Neoplastic effects	None	Mononuclear cell leukemia (8/50, 11/50, 15/50, 16/50)	None	None
Level of evidence of carcinogenic activity	No evidence	Equivocal evidence	No evidence	No evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **clear evidence** and **some evidence**; one category for uncertain findings **equivocal evidence**; one category for no observable effects **no evidence**; and one category for experiments that because of major flaws cannot be evaluated **inadequate study**. These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.

- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.

- **Inadequate study** of carcinogenic activity describes studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the NTP draft Technical Report on chlorinated and chloraminated water on November 19, 1990 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D., Chair
Medicine and Environmental Health Department
Research and Environmental Health Division, Exxon Corp.
East Millstone, NJ

Jay I. Goodman, Ph.D., Principal Reviewer
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

Daniel S. Longnecker, M.D., Principal Reviewer
Department of Pathology
Dartmouth Medical School, Hanover, NH

Ellen K. Silbergeld, Ph.D.
University of Maryland Medical School
Baltimore, MD

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D., Principal Reviewer
Central Toxicology Laboratory
Imperial Chemical Industries, PLC
Alderly Park, England

Gary P. Carlson, Ph.D.
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Harold Davis, D.V.M., Ph.D.
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, D.V.M.
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D.
Lawrence Berkeley Laboratory
University of California
Berkeley, CA

David W. Hayden, D.V.M., Ph.D.
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Curtis D. Klaassen, Ph.D.
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Barbara McKnight, Ph.D.
Department of Biostatistics
University of Washington
Seattle, WA

Lauren Zeise, Ph.D.
California Department of Health Services/RCHAS
Berkeley, CA

SUMMARY OF PEER REVIEW COMMENTS

On November 19, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of chlorinated and chloraminated water received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of chlorinated and chloraminated water by discussing the uses of chlorine and chloramine, experimental design, survival and water consumption in rats and mice, and results. The proposed conclusions were that there was *no evidence of carcinogenic activity* of chlorinated water for male F344/N rats or male or female B6C3F$_1$ mice and *equivocal evidence of carcinogenic activity* of chlorinated water for female F344/N rats. There was *no evidence of carcinogenic activity* of chloraminated water for male F344/N rats or male or female B6C3F$_1$ mice and *equivocal evidence of carcinogenic activity* of chloraminated water for female F344/N rats.

Dr. Longnecker, the first principal reviewer, agreed with the conclusions but asked for more discussion of the rationale for *equivocal evidence* in female rats. He asked why there was such low survival in dosed and control male rats and what impact this low survival might have had on the validity of the studies. Dr. S. Eustis, NIEHS, commented that higher incidences of leukemias, pituitary gland tumors, and kidney disease contributed to lower survival in male rats in these studies as well as in other more recent studies.

Dr. Goodman, the second principal reviewer, agreed with the conclusions in male rats and male and female mice, but did not agree with the conclusions in female rats, which he recommended be changed to *no evidence of carcinogenic activity*. He cited the high and variable incidence of leukemias in historical controls, noting that the incidences of leukemias in treated groups in these studies were within the range of historical controls, the relatively low incidence in concurrent controls, and the lack of a dose-response relationship. He further noted that the statistical significance ($P<0.05$) was marginal for such commonly occurring neoplasms. Dr. Dunnick responded by saying that emphasis is given primarily to concurrent control values.

Dr. Ashby, the third principal reviewer, agreed with the conclusions. He commented that because the water used had been treated with activated carbon and deionized prior to chlorination, a more descriptive title than "chlorinated drinking water" might be appropriate. Dr. Dunnick said text would be added to the Abstract and elsewhere to point out that the studies were intended to determine the toxicity and carcinogenicity of chlorinated or chloraminated water without the confounding effects of byproducts. Dr. Ashby expressed concern about the survival of male rats and its effect on the adequacy of the studies. Dr. J. Haseman, NIEHS, said that, in the judgment of the NTP, survival of male rats was sufficient to permit an evaluation of carcinogenicity.

There was some debate over whether discussion of the effects of trihalomethanes should be included, as the water purification processes would have removed any of these chemicals present. Dr. Silbergeld questioned the relevance to human exposure. There was also considerable discussion about the variability and increasing incidence of mononuclear cell leukemias in rats and how this affected the interpretation of the findings in dosed female rats.

Dr. Longnecker moved that the draft Technical Report on the studies of chlorinated and chloraminated water be accepted with the revisions discussed, including a modification of the report title and of the description of what was tested, and with the conclusions as written for male rats and male and female mice, *no evidence of carcinogenic activity*, and for female rats, *equivocal evidence of carcinogenic activity*. Dr. Ashby seconded the motion. Dr. Zeise offered an amendment that the studies of chlorinated water in male rats be considered an *inadequate study of carcinogenic activity* due to poor survival and inadequate dosing. Dr. Silbergeld seconded the motion, which was defeated by ten votes to two (Drs. Silbergeld, Zeise). Dr. Goodman offered an amendment that the conclusions in female rats be changed to *no evidence of carcinogenic activity*. Dr. Carlson seconded the motion, which

was defeated by nine votes to three (Drs. Carlson, Gold, Goodman). The original motion by Dr. Longnecker was then accepted by eight votes to three (Drs. Carlson, Gold, Goodman), with one abstention (Dr. Silbergeld).